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Dielectric Behavior of Liposomes of Large Size

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By using liposomes prepared by the method of reverse-phase evaporation, dielectric behavior was studied of the liposomes of large size. The liposomes showed a marked dielectric relaxation with the distribution of relaxation frequencies. From the dielectric observations on filtered specimens, it was inferred that the distribution of relaxation frequencies was not caused by the distribution of the diameter of suspended particles. Assuming that the electrical conductivity of the shell phase of the liposomes is much lower than that of the outer medium and that of the inner phase, observed data were analyzed in the light of a theory of interfacial polarization in suspensions of shelled spheres. Relative permittivities and electrical conductivities of the shell phase and the inner phase were calculated by using formulas derived from the theory. The specific membrane capacitance of the shell phase was estimated to be 1.3 μF cm^{-2}. Liposomes of small size were produced by sonication. Dielectric properties of the suspend ed particles of the liposomes remained unchanged regardless of dilution. The values of the volume fraction of diluted specimens were consistent with the dilutions in the preparation of specimens. Under different osmolarity in the outer medium varied by changing glucose or KCl concentration, the volume of suspended particles changed following the van't Hoff equation. From this result, the shell phase is seen to be a semipermeable membrane through which water can permeate but glucose and KCl cannot. The conductivity of the inner phase was linearly proportional to that of the outer medium.

KEY WORDS: Conductivity/ Dielectric property/ Interfacial polarization/ Liposomes/ Permittivity/

I. INTRODUCTION

Colloid and coarse dispersions show dielectric behavior characteristic of their structures.[1-3] In many instances, the dielectric behavior of the dispersions was interpreted in terms of theories of interfacial polarization. From the theories of interfacial polarization, it was deduced that W/O/W emulsions exhibited a couple of dielectric relaxations.[4,5] In certain cases, only one dielectric relaxation was observed because one of the relaxations was much larger than the other.

Natural products in the W/O/W type such as biological cells and subcellular organella exhibited remarkable dielectric relaxations.[6,7] Membrane capacitance, permittivity and conductivity of the cell interior were evaluated from these dielectric data on the basis of the theory of interfacial polarization.

In contrast to these examples, few dielectric measurements have been carried out so far for artificial W/O/W emulsions.[8] Several techniques to obtain stable W/O/W emulsions of large size were reported recently by many workers.[9-13] Zhang *et al.* reported dielectric observations on suspensions of polystyrene microcapusules,[14] which were prepared by means of the interfacial polymer deposition technique.[13]

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In this paper, dielectric behavior is reported of liposomes of large size prepared by the method of reverse-phase evaporation.[15,16] The liposomes show remarkable dielectric relaxations, which are characteristic of the treatments for the specimens. The observed data are analyzed in the light of the theory of interfacial polarization.

II. THEORETICAL

1. Theoretical Formulas for a Suspension of Shelled Spheres

Liposomes are considered to be a suspension in which spheres (the complex relative permittivity ε_i^*) covered with a shell phase (ε_s^*, and the thickness d) are suspended in an outer medium (ε_a^*) with a volume fraction Φ.

According to the previous paper,[5] the complex permittivity of such a suspension (ε^*) is given by

$$\frac{\varepsilon^*-\varepsilon_q^*}{\varepsilon_a^*-\varepsilon_q^*}\left(\frac{\varepsilon_a^*}{\varepsilon^*}\right)^{1/3}=1-\Phi, \tag{1}$$

where ε_q^* is the equivalent complex permittivity of the shelled sphere (the outer diameter D), being given by

$$\varepsilon_q^*=\varepsilon_s^*\frac{2(1-v)\varepsilon_s^*+(1+2v)\varepsilon_i^*}{(2+v)\varepsilon_s^*+(1-v)\varepsilon_i^*}, \tag{2}$$

and

$$v=\left(1-\frac{2d}{D}\right)^3. \tag{3}$$

The complex permittivities ε^*, ε_i^*, ε_s^*, ε_a^* and ε_q^* are defined by an equation of the following form with subscripts i, s, a and q.

$$\varepsilon^*=\varepsilon-j\frac{\kappa}{2\pi f\epsilon_v}, \tag{4}$$

where ε, j, κ, ϵ_v and f are the relative permittivity, imaginary unit $\sqrt{-1}$, the electrical conductivity, the permittivity of vacuum and the frequency, respectively.

From Eqs. (1) and (2), the suspension of shelled spheres is seen to exhibit two dielectric relaxations termed P-relaxation for lower frequencies and Q-relaxation for higher frequencies. Limiting values of relative permittivity ε_l and ε_h and electrical conductivity κ_l and κ_h at low (subscript l) and high (h) frequencies are given by

$$\varepsilon_l\left(\frac{3}{\kappa_l-\kappa_{ql}}-\frac{1}{\kappa_l}\right)=3\left(\frac{\varepsilon_a-\varepsilon_{ql}}{\kappa_a-\kappa_{ql}}+\frac{\varepsilon_{ql}}{\kappa_l-\kappa_{ql}}\right)-\frac{\varepsilon_a}{\kappa_a}, \tag{5}$$

$$\frac{\varepsilon_h-\varepsilon_{qh}}{\varepsilon_a-\varepsilon_{qh}}\left(\frac{\varepsilon_a}{\varepsilon_h}\right)^{1/3}=1-\Phi, \tag{6}$$

$$\frac{\kappa_l-\kappa_{ql}}{\kappa_a-\kappa_{ql}}\left(\frac{\kappa_a}{\kappa_l}\right)^{1/3}=1-\Phi, \tag{7}$$

and

$$\kappa_h\left(\frac{3}{\varepsilon_h-\varepsilon_{qh}}-\frac{1}{\varepsilon_h}\right)=3\left(\frac{\kappa_a-\kappa_{qh}}{\varepsilon_a-\varepsilon_{qh}}+\frac{\kappa_{qh}}{\varepsilon_h-\varepsilon_{qh}}\right)-\frac{\kappa_a}{\varepsilon_a}, \tag{8}$$

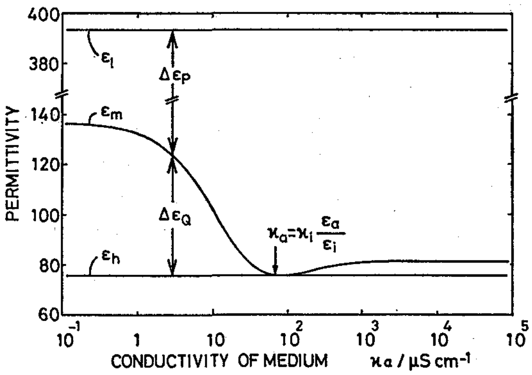

Fig. 1. Change in the values of the relative permittivity ε_m at intermediate frequency and the limiting values ε_l and ε_h at low and high frequencies with the change in the conductivity κ_a in the outer medium. They were calculated from Pauly-Schwan's theory using the follwoing phase parameters : $\varepsilon_i=70$, $\kappa_i=60$ μS cm^{-1}, $\varepsilon_s=7$, $\kappa_s=0$ S cm^{-1}, $\varepsilon_a=80$, $D=1$ μm, $d=5$ nm and $\Phi=0.26$.

where ε_{ql}, ε_{qh}, κ_{ql} and κ_{qh} are the limiting values at low and high frequencies of the equivalent relative permittivity and the equivalent electrical conductivity of the shelled sphere. They are given by

$$\varepsilon_{ql}=\varepsilon_s\frac{\kappa_{ql}}{\kappa_s}+\frac{9(\varepsilon_i\kappa_s-\varepsilon_s\kappa_i)\kappa_s v}{[(2+v)\kappa_s+(1-v)\kappa_i]^2}, \tag{9}$$

$$\varepsilon_{qh}=\varepsilon_s\frac{2(1-v)\varepsilon_s+(1+2v)\varepsilon_i}{(2+v)\varepsilon_s+(1-v)\varepsilon_i}, \tag{10}$$

$$\kappa_{ql}=\kappa_s\frac{2(1-v)\kappa_s+(1+2v)\kappa_i}{(2+v)\kappa_s+(1-v)\kappa_i}, \tag{11}$$

and

$$\kappa_{qh}=\kappa_s\frac{\varepsilon_{qh}}{\varepsilon_s}+\frac{9(\kappa_i\varepsilon_s-\kappa_s\varepsilon_i)\varepsilon_s v}{[(2+v)\varepsilon_s+(1-v)\varepsilon_i]^2}. \tag{12}$$

2. Behavior of the Intensity of the P-relaxation and the Q-relaxation

In order to examine the behavior of the intensity of the P-relaxation $\Delta\varepsilon_P$ and the Q-relaxation $\Delta\varepsilon_Q$, relative permittivity ε_m at intermediate frequencies, ε_l and ε_h were calculated by Pauly-Schwan's theory,[4] which is valid in dilute suspensions. Figure 1 shows the change in ε_l, ε_m and ε_h with the change in κ_a. The value of $\Delta\varepsilon_P$ is given by $\Delta\varepsilon_P=\varepsilon_l-\varepsilon_m$, while $\Delta\varepsilon_Q$ is given by $\Delta\varepsilon_Q=\varepsilon_m-\varepsilon_h$. In the case of $\kappa_a<\kappa_i$, $\Delta\varepsilon_Q$ is in the same order of magnitude as that of $\Delta\varepsilon_P$. In the case of $\kappa_a>\kappa_i$, $\Delta\varepsilon_Q$ turns out negligibly small compared with $\Delta\varepsilon_P$.

III. EXPERIMENTAL

1. Preparation of Liposomes

Liposomes were prepared by the method of reverse-phase evaporation reported by Szoka, Jr. *et al.*.[15,16] A mixture of egg lecithin (Merck) and cholesterol (Standard for clinical work, Wako Pure Chemical Industries) in molar ratio 1 : 1 was used for the preparation of the liposomes. Aqueous solutions prepared for the inner phase of the liposomes contain KCl and glucose, whose concentrations were controlled to adjust the electrical conductivity and the osmolarity of the inner phase. Ficoll-400 was also added to the inner phase in 17 wt.% to facilitate the sedimentation of the suspended particles in the specimen. By means of centrifugation, the liposomes prepared were washed with aqueous solutions whose composition was the same as those used for the inner phase of the liposomes except for absence of Ficoll-400.

2. Dielectric Measurements

Capacitance and conductance were measured with a TR-1BK Transformer Ratio Arm Bridge made by Ando Electric Co., Ltd. over a frequency range 100 Hz to 1 MHz, and with a Model 4191A RF Impedance Analyser made by Hewlett-Packard Co., Ltd. over a range 1 to 200 MHz.

A measuring cell consisted of two concentric platinum cylinders, which was the same as used in a previous paper.[17] The cell constant of 1.16 pF was determined by using air and several standard liquids.

The dielectric measurements were carried out at 25°C. Observed data were subjected to corrections[18,19] for the errors arising from residual inductance caused by the cell assembly. The increase in capacitance due to electrode polarization was corrected by use of the observed data at low frequencies.[20]

IV. RESULTS AND DISCUSSION

1. Dielectric Observations on the Liposomes and Estimation of Phase Parameters from Dielectric Parameters Observed

Figure 2(A) shows the frequency dependence of the relative permittivity ε and the electrical conductivity κ for the liposomes prepared with a 1 mM KCl solution for both the inner phase and the outer medium. Complex plane plots of the same data are shown in Fig. 2(B). As seen in Fig. 2(A) and Fig. 2(B), the liposomes showed a marked dielectric relaxation with the distribution of relaxation frequencies. Limiting values of the permittivity ε_l and ε_h at low and high frequencies were obtained by extrapolating the observed data to low and high frequencies with circular arcs on the complex plane plots, respectively. In this paper, the relaxation frequency f_0 was defined as the frequency corresponding to $\varepsilon=(\varepsilon_l+\varepsilon_h)/2$. Following the definition, the value of f_0 was determined graphically.

By the use of observed values of the dielectric parameters such as κ_l, ε_l, ε_h and f_0, the values of the phase parameters such as the volume fraction Φ, the relative permittivity ε_s of the shell phase, the relative permittivity ε_i and the electrical conductivity κ_i of the inner phase were estimated by means of a curve fitting method reported in the previous paper.[5] Since conductivities of lipid membranes are very low in comparison with the aqueous phase, the electrical conductivity κ_s of the shell phase is assumed to be much lower than that of the inner phase κ_i and that of the outer medium κ_a. Phase parameters estimated from the data shown in Fig. 2 are listed in Table I. The relative permittivity ε_a and the electrical conductivity κ_a of the outer medium were obtained by the dielectric measurement of the supernatant after centrifuging the liposomes at $2000\times g$ for 2 h.

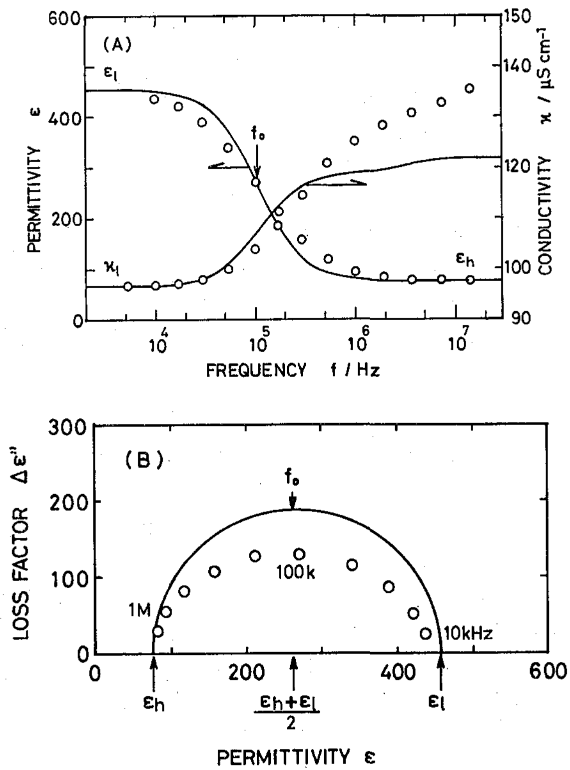

Fig. 2. (A) Frequency dependence of the relative permittivity ε and the electrical conductivity κ, and (B) the complex plane plots of the dielectric relaxation for the liposomes prepared with a 1 mM KCl solution for both the inner phase and the outer medium. The solid curves are calculated from Eqs. (1) and (2) by using the phase parameters obtained.

The value of κ_i ($=58\,\mu$S cm^{-1}) for the present liposomes was lower than κ_a ($=151\,\mu$S cm^{-1}). In this case, only the P-relaxation can be observed because of $\Delta\varepsilon_Q \ll \Delta\varepsilon_P$ as discussed in Section II-2. The relaxation frequency f_0 observed in Fig. 2 is interpreted as that of the P-relaxation.

Table I. The phase parameters calculated for the liposomes prepared with a 1 mM KCl solution and the change in the values of the phase parameters with the change in D/d

D / μm	d / nm	D/d	Phase Parameter Calculated: Φ	ε_s	DC_M / pF cm^{-1}	ε_i	κ_i / μS cm^{-1}
2	5	400	0.258	3.74	132	69.8	57.7
1	5	200	0.258	7.51	133	70.0	58.0
0.5	5	100	0.258	15.2	135	70.3	58.5

Dielectric parameters observed: $\varepsilon_l=454$, $\varepsilon_h=75.6$, $\kappa_l=96.5\,\mu$S cm^{-1}, $f_0=106$ kHz.
Outer medium: $\varepsilon_a=80.1$, $\kappa_a=151\,\mu$S cm^{-1}.

The change in the values of phase parameters with the change in D/d is summarized in Table I. Since the values of ε_s are inversely proportional to D/d, the capacitive property of the shell phase is appropriately represented by DC_M (the product of the outer diameter D of the shelled spheres and the specific membrane capacitance C_M of the shell phase given by $C_M=\varepsilon_s\epsilon_v/d$). Other phase parameters such as Φ, ε_i and κ_i are seen to be independent of the values of D/d.

According to electron microscopic observation, the mean diameter of the suspended particles was 1.0 μm. If we assume $D=1.0\ \mu$m, C_M can be evaluated from the value of DC_M to be 1.3 μF cm^{-2}, which is consistent with those of biological cells (about 1 μF cm^{-2}). The values of ε_i are close to the relative permittivity of water. The values of κ_i are about 1/3 of the conductivity of 1 mM KCl solutions.

2. Effect of Filtration

Liposomes were prepared with a 200 mM glucose solution for both the inner phase and the outer medium of the liposomes. They were fractionated by use of filters (Uni-Pore membrane filter, Bio-Rad Laboratories) of the pore size 1 μm and 0.6 μm. Before the dielectric measurements, the specimens were concentrated by centrifuging at $2000\times g$ for 2 h. In the case of the specimen sieved through the filter of the pore size 0.6 μm, the precipitate was loosely packed compared with the other specimens. The values of ε_a and κ_a were obtained by the dielectric measurements of the supernatants.

The results of dielectric measurements of a control specimen and the fractionated specimens are shown in Fig. 3 and Table II. As seen in the figure, the observed data are represented satisfactorily by circular arcs given by

$$\varepsilon-j\frac{\kappa}{2\pi f\epsilon_v}=\varepsilon_h+\frac{\varepsilon_l-\varepsilon_h}{1+[j(f/f_0')]^\beta}-j\frac{\kappa_l}{2\pi f\epsilon_v}\ ;\ 0<\beta\leqq 1, \tag{13}$$

where f_0' is the most probable relaxation frequency and β is the distribution parameter of relaxation frequencies.[21]

According to electron microscopic observation, the diameter of the suspended particles was distributed over a range of 0.1 μm to 3 μm. If the distribution of relaxation frequencies is caused by the distribution of the diameter of the suspended particles, the dielectric relaxation is to tend toward the system with a single relaxation time as the liposomes approach a monodispersion, giving the value of β closer to

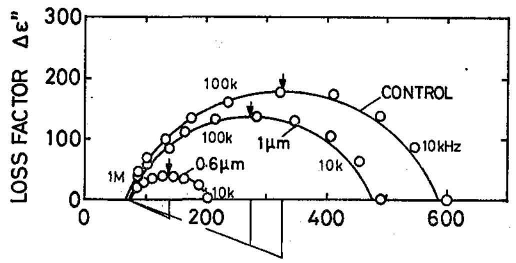

Fig. 3. The complex plane plots of the dielectric relaxation for the filtered liposomes. The solid curves are circular arcs given by Eq. (13). The relaxation frequencies of each specimen are represented by arrows.

Table II. The dielectric parameters observed and the phase parameters calculated for the liposomes fractionated by use of filters

Filter	Outer Medium		Dielectric Parameter Observed					Phase Parameter Calculated			
Pore Size μm	ε_a	$\frac{\kappa_a}{\mu\text{S cm}^{-1}}$	ε_l	ε_h	$\frac{\kappa_l}{\mu\text{S cm}^{-1}}$	$\frac{f_0}{\text{kHz}}$	β[a]	Φ	$\frac{DC_M}{\text{pF cm}^{-1}}$	ε_i	$\frac{\kappa_i}{\mu\text{S cm}^{-1}}$
Control	78.8	29.6	586	68.9	18.5	51.5	0.76	0.269	171	49	58
1	78.3	30.8	475	69.0	19.2	60	0.76	0.270	135	51	50
0.6	77.4	40.5	202	73.1	33.3	77	0.74	0.122	93	51	35

a) The distribution parameter of relaxation frequencies β is given in Eq. (13).

unity. In order to make the diameter of the suspended particles uniform, large particles suspended were removed by filtration. In spite of the unification of the particle size, the values of β shown in Table II remain unchanged regardless of the filtration treatment. This result suggests that the distribution of relaxation frequencies is not caused by the distribution of the diameter of the suspended particles.

Provided that the value of C_M is 1.3 μF cm^{-2}, the values of D can be estimated from the values of DC_M to be 1.3 μm for the control, 1.0 μm and 0.7 μm for the two specimens sieved through the filters of the pore size 1 μm and 0.6 μm. The decrease in the values of D by the filtration reflects the fact that the mean diameter of suspended particles is decreased by the filtration.

3. Effect of Sonication

The liposomes were sonicated at 0°C. The frequency dependence of ε and κ of the control specimen and those sonicated for 5 and 30 min are illustrated in Fig. 4. Phase

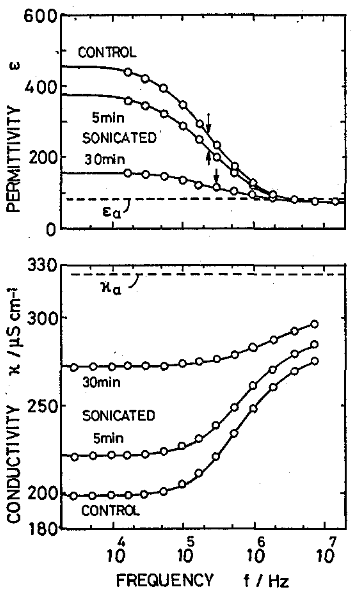

Fig. 4. Frequency dependence of ε and κ for the sonicated liposomes. The relaxation frequencies of each specimen are represented by arrows.

Table III. The dielectric parameters observed and the phase parameters calculated for the sonicated liposomes

Sonication	Dielectric Parameter Observed				Phase Parameter Calculated			
Time / min	ε_l	ε_h	κ_l / μS cm^{-1}	f_0 / kHz	Φ	DC_M / pF cm^{-1}	ε_i	κ_i / mS cm^{-1}
Control	456	75.1	198	225	0.281	124	70	0.11
5	376	75.2	221	225	0.227	120	66	0.11
30	155	76.0	273	280	0.110	65	54	0.071

Outer medium: $\varepsilon_a=80.2$, $\kappa_a=325\ \mu$S cm^{-1}.

parameters evaluated from these data are listed in Table III. The sonicated liposomes were hardly precipitated by the centrifugation. Hence, the values of ε_a and κ_a were obtained by the dielectric measurement of the supernatant after the centrifugation of the liposomes which were not sonicated.

The values of ε_l decreased and those of κ_l increased with the sonication. These changes in dielectric parameters seem to result from the decrease in Φ and DC_M. If we assume that C_M remains unchanged by the sonication, the decrease in DC_M suggests the decrease in D. As reported by Huang,[22] liposomes of small size (mean diameter: about 25 nm) were produced by sonication of lipid suspended in water. Provided that the liposomes of small size are produced by the sonication of the liposomes of large size, the volume fraction Φ and the diameter D are to decrease by the sonication. From the present results of the dielectric measurements on the sonicated liposomes, it is suggested that the liposomes of small size are produced by sonicating the liposomes of large size.

4. Change in Volume Fraction

The liposomes prepared with a 2 mM KCl solution for both the inner phase and the outer medium were kept in a cellulose tube for dialysis (Visking) and stored overnight at room temperature in an aqueous solution of the same composition as the outer medium. The liposomes were then diluted with the medium used in the dialysis. The values of ε_a and κ_a were obtained by the dielectric measurements of the medium used in

Table IV. The dielectric parameters observed and the phase parameters calculated for the liposomes in different dilutions

Dilution	Dielectric Parameter Observed				Phase Parameter Calculated				
	ε_l	ε_h	κ_l / μS cm^{-1}	f_0 / kHz	Φ	Φ_R [a]	DC_M / pF cm^{-1}	ε_i	κ_i / mS cm^{-1}
1	652	71.4	132	208	0.452	100	124	68	0.11
5/4	558	73.1	164	220	0.366	81	124	68	0.11
5/3	456	75.1	198	225	0.281	62	124	70	0.11
5/2	335	77.0	238	240	0.188	42	123	71	0.12

Outer medium: $\varepsilon_a=80.2$, $\kappa_a=325\ \mu$S cm^{-1}.

a) The relative volume fraction Φ_R is defined as the ratio of the volume fraction of the diluted specimens to that of the undiluted specimen.

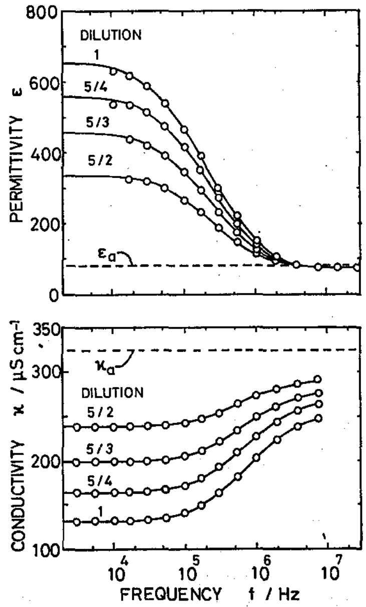

Fig. 5. Frequency dependence of ε and κ for the liposomes in different dilutions.

the dialysis.

Figure 5 and Table IV show the results of the dielectric measurements of the liposomes in different dilutions. The volume fraction Φ decreased with the dilution of the liposomes. Relative volume fraction Φ_R is defined as the ratio of the volume fraction of the diluted specimens to that of the undiluted specimen. The values of Φ_R shown in Table IV are consistent with the dilutions in the preparation of specimens. Other phase parameters, DC_M, ε_i and κ_i remain unchanged regardless of the dilution. These results are reasonable because these phase parameters should be inherent in the suspended particles themselves and be independent of the volume fraction.

5. Change in Osmolarity in the Outer Medium

The dielectric measurements of the liposomes in different osmolarity in their outer medium were carried out by the following procedure.

Step 1 Liposomes prepared with a solution whose osmolarity was adjusted to C_b were divided into several fractions in equal volume. The volume of each of the fractions was V_b.

Step 2 Solutions whose osmolarity was adjusted to C_x were added to each of the fractions up to equal volume V_t. At this stage, the osmolarity in outer medium was C_a. The values of C_a were varied by changing C_x.

Step 3 Dielectric measurements of each of the fractions were performed within one hour after changing the osmolarity.

Step 4 The values of ε_a and κ_a were obtained from dielectric measurements of the supernatant of each of the fractions after centrifugation.

Liposomes were prepared with a 1 mM KCl solution whose osmolarity was adjusted to 20 mOsm by adding glucose. The unit of osmolarity Osm means Osmol l^{-1}. The

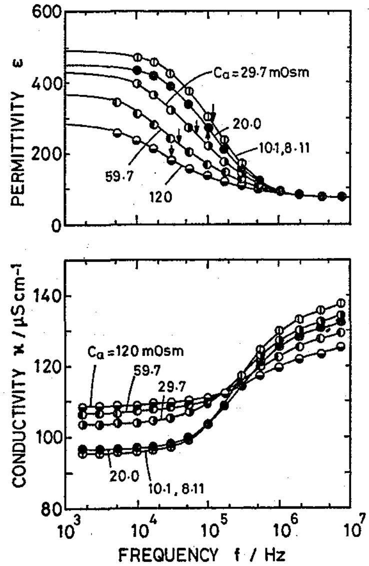

Fig. 6. Frequency dependence of ε and κ for the liposomes with different osmolarity in the outer medium C_a. The liposomes were prepared with a 20 mOsm solution, C_a being changed by using glucose.

osmolarity in their outer medium was changed by using glucose. Figure 6 and Table V show the results of the dielectric measurements in which the osmolarity in the outer medium was changed by using glucose. By use of the liposomes prepared with a 2 mM KCl solution (its osmolarity was 4 mOsm), a series of measurements was carried out in which the osmolarity in the outer medium was changed by use of KCl. Their results are given in Fig. 7 and Table VI.

The change in the volume of the suspended particles was analyzed in the light of the

Table V. The dielectric parameters observed and the phase parameters calculated for the liposomes prepared with a 20 mOsm solution in different osmolarity in the outer medium changed by using glucose

Outer Medium			Dielectric Parameter Observed				Phase Parameter Calculated				
C_a a) / mOsm b)	ε_a	κ_a / μS cm^{-1}	ε_l	ε_h	κ_l / μS cm^{-1}	f_0 / kHz	Φ	V_R c)	DC_M / pF cm^{-1}	ε_i	κ_i / μS cm^{-1}
8.11	79.8	156	488	75.5	95	119	0.282	1.10	134	72	67
10.1	79.7	157	496	75.3	96	116	0.280	1.09	137	71	67
20.0	80.1	151	454	75.6	97	106	0.256	1.00	134	70	59
29.7	79.9	144	431	75.8	104	68	0.195	0.76	160	65	42
59.7	79.8	136	372	76.2	107	39	0.148	0.58	173	61	24
120	79.6	133	287	76.4	109	31	0.124	0.48	147	61	16

a) The osmolarity in the outer medium.
b) The unit of osmolarity Osm means Osmol l^{-1}.
c) The relative particle volume V_R is defined as the ratio of the volume of the suspended particles in changed osmolarity to that under isotonic condition.

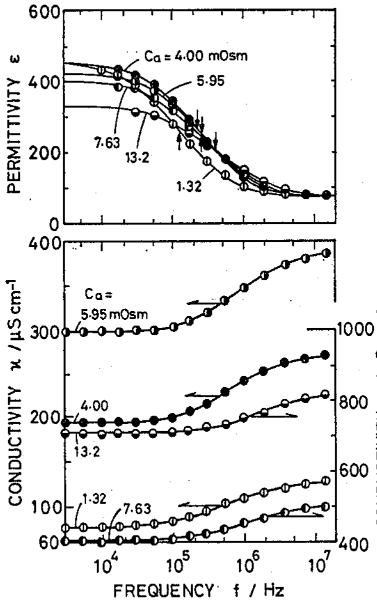

Fig. 7. Frequency dependence of ε and κ for the liposomes with different C_a. The liposomes were prepared with a 4 mOsm solution, C_a being changed by using KCl.

van't Hoff equation. If the shell phase of the liposomes possesses the nature of a semi-permeable membrane, the volume V_q of the suspended particles changes following the relation,

$$V_q = \frac{A}{C_a} + V_d, \tag{14}$$

where A is a constant independent of V_d, C_a the osmolarity in the outer medium and V_d the osmotically dead volume of the suspended particles.

In our experiments, the volume of the suspended particles is represented by relative particle volume V_R in place of V_q. The V_R is defined as the ratio of V_q under changed

Table VI. The dielectric parameters observed and the phase parameters calculated for the liposomes prepared with a 4 mOsm solution in different osmolarity in the outer medium changed by using KCl

Outer Medium			Dielectric Parameter Observed				Phase Parameter Calculated				
C_a / mOsm	ε_a	κ_a / μS cm^{-1}	ε_l	ε_h	κ_l / μS cm^{-1}	f_0 / kHz	Φ	V_R	DC_M / pF cm^{-1}	ε_i	κ_i / mS cm^{-1}
1.32	79.5	122	452	75.7	76.8	122	0.265	1.03	129	73	0.071
4.00	79.4	306	450	75.7	196	222	0.257	1.00	132	73	0.12
5.95	79.4	443	419	75.7	298	268	0.232	0.90	133	71	0.14
7.63	79.8	558	401	75.9	406	268	0.191	0.74	150	66	0.15
13.2	79.5	943	329	76.3	712	402	0.171	0.67	131	68	0.20

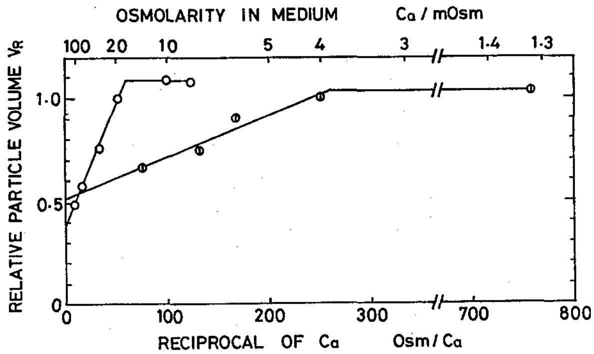

Fig. 8. Plots of relative particle volume V_R against reciprocal of C_a. The value of C_a was changed by using glucose (○) or KCl (⦶).

osmolarity to V_{q0}, which is the value of V_q under isotonic condition. The value of V_R is evaluated by

$$V_R = \frac{\Phi}{\Phi_0}, \tag{15}$$

where Φ_0 is the volume fraction under the isotonic condition. From Eq. (14), V_R varies in the manner given by

$$V_R = \frac{A}{V_{q0}C_a} + \frac{V_d}{V_{q0}}. \tag{16}$$

On the other hand, the value of C_a can be evaluated by

$$C_a = \frac{C_b(V_b - \Phi_0 V_t) + C_x(V_t - V_b)}{V_t(1-\Phi)}. \tag{17}$$

In Fig. 8, V_R is plotted against the reciprocal of C_a. Under hypertonic condition ($C_a > C_b$), the suspended particles shrink in the manner following Eq. (16). This result suggests that the shell phase of the liposomes is a semipermeable membrane through which water can permeate but glucose and KCl cannot. The volume of the suspended particles remain unchanged under hypotonic condition ($C_a < C_b$). It seems that the shell phase resists the expansion under hypotonic condition.

6. Change in Electrical Conductivity in the Outer Medium and the Inner Phase

Liposomes were prepared with solutions whose osmolarity was adjusted to 200 mOsm by adding glucose. The values of κ_i and κ_a were varied by changing KCl concentration. Figures 9 and 10 show the results of the dielectric measurements in different κ_a under both isotonic and hypotonic conditions. The osmolarity in their outer medium was controlled by using glucose. The results obtained from the liposomes prepared with a 1 mM KCl solution are given in Fig. 9 and those obtained from the liposomes prepared with a 10 mM KCl solution are given in Fig. 10. Phase parameters evaluated from these data are summarized in Table VII. The values of ε_a and κ_a were obtained by the dielectric measurements of the supernatants after centrifuging the liposomes.

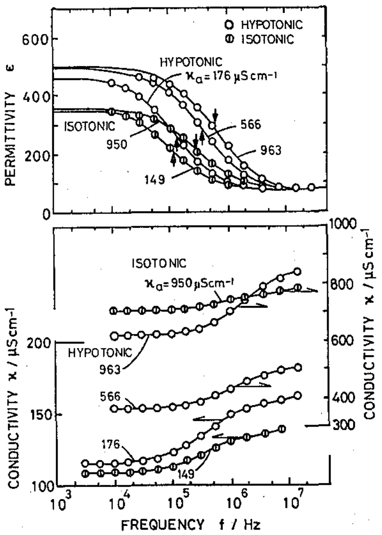

Fig. 9. Frequency dependence of ε and κ for the liposomes prepared with a 1 mM KCl solution in different κ_a.

The values of ε_l and ε_h remained unchanged regardless of the change in κ_a. The relaxation frequency f_0 shifted to higher frequencies with the increase in κ_a. The values of Φ, DC_M and ε_i remained unchanged regardless of the change in κ_a, whereas the values of κ_i were dependent on κ_a. Figure 11 shows the relationship between κ_i and κ_a. The values of κ_i are proportional to κ_a, being independent of the concentration of KCl in the inner phase. The solid line in Fig. 11 is the empirical regression curve which is given by

$$\kappa_i/\text{mS cm}^{-1}=0.017+0.293\,\kappa_a/\text{mS cm}^{-1}. \qquad (18)$$

Since the shell phase of the liposomes is impermeable to KCl as discussed in the

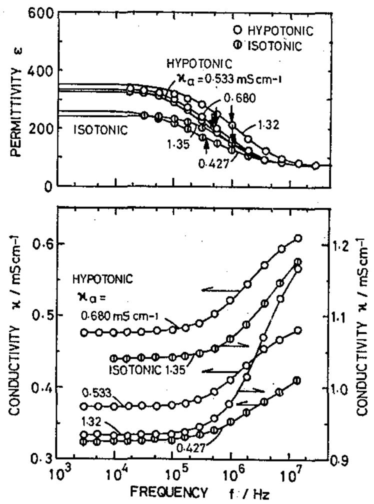

Fig. 10. Frequency dependence of ε and κ for the liposomes prepared with a 10 mM KCl solution in different κ_a.

Table VII. The dielectric parameters observed and the phase parameters calculated for the liposomes prepared with 200 mOsm solutions in different conductivity in the outer medium changed by using KCl

Inner Phase	Outer Medium			Dielectric Parameter Observed				Phase Parameter Calculated			
KCl mM	C_a mOsm	κ_a mS cm^{-1}	ε_a	ε_l	ε_h	κ_l mS cm^{-1}	f_0 MHz	Φ	DC_M pF cm^{-1}	ε_i	κ_i mS cm^{-1}
1	200	0.950	78.9	354	75.7	0.707	0.270	0.179	138	68	0.13
1	200	0.149	78.6	344	75.3	0.108	0.114	0.193	124	69	0.058
1	71	0.963	79.3	498	75.6	0.622	0.580	0.253	151	71	0.35
1	71	0.566	79.2	496	75.3	0.366	0.350	0.252	150	70	0.21
1	71	0.176	79.1	455	74.7	0.115	0.122	0.247	139	68	0.070
10	200	1.35	78.6	240	75.3	1.04	1.06	0.160	93	68	0.39
10	200	0.427	78.9	258	75.6	0.324	0.350	0.168	98	69	0.14
10	76	1.32	78.6	353	74.8	0.934	0.980	0.206	121	68	0.48
10	76	0.680	78.8	333	74.7	0.478	0.505	0.209	111	67	0.23
10	76	0.533	78.6	323	74.5	0.373	0.440	0.212	106	68	0.19

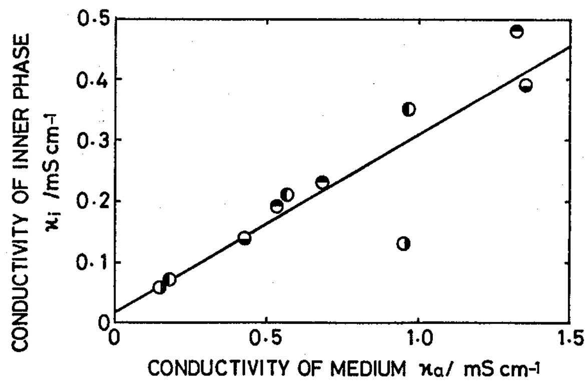

Fig. 11. Plots of the electrical conductivity in the inner phase κ_i against κ_a. The κ_a of the liposomes prepared with the 1 mM KCl solution was changed under isotonic condition (◑) and under hypotonic condition (◐). The κ_a of the liposomes prepared with the 10 mM KCl solution was changed under isotonic condition (◒) and under hypotonic condition (◓). The solid line is the regression curve given by Eq. (18).

preceding section, the values of κ_i are expected to remain unchanged regardless of the change in κ_a. At the same time, the values of κ_i of the liposomes prepared with the 10 mM KCl solution should be ten times higher than those of the liposomes prepared with the 1 mM KCl solution. The cause of these anomalous observations on κ_i described above is not understood yet.

V. CONCLUSIONS

1. The value of C_M was 1.3 μF cm^{-2}, which is consistent with those of biological cells.

2. The values of β remained unchanged regardless of the filtration of the liposomes. This result suggests that the distribution of relaxation frequencies is not caused by the

distribution of the diameter of the suspended particles.

3. Both Φ and DC_M decreased by the sonication. It is suggested from this fact that the liposomes of small size are produced by the sonication.

4. The values of DC_M, ε_i and κ_i remained unchanged regardless of the dilution of the liposomes. The values of Φ_R are consistent with the dilutions in the preparation.

5. Under different osmolarity in the outer medium changed by using glucose or KCl, the change in V_R was successfully represented by the van't Hoff equation. It is suggested from this fact that the shell phase is a semipermeable membrane through which water can permeate but glucose and KCl cannot.

6. The κ_i was linearly proportional to κ_a.

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